SECTION : 4

INTRODUCTION, DISCUSSION

AND

EXPERIMENTAL

TO

QUALITATIVE AND QUANTITATIVE

DETERMINATION OF CARBOHYDRATES PRESENT

IN SOME MEDICINAL PLANTS BY

PAPER CHROMATOGRAPHY
Systematic investigation of drugs used in indigenous medicine in India on modern scientific lines was started recently. The constituents have been examined and preparations made from the medicinal plants have been tested. It is only by a thorough enquiry that the merits of these medicinal plants can be proved. Most of the medicinal plants used in indigenous medicine are supposed to be specified for particular disease. There is a clear need for systematic research on these Indian medicinal plants.

While it is not our object here to consider the merits of such revival, we have no doubt that out of the large number of drugs used by the "Kavirajes" and "Hakims" for centuries past and still in use, there are many that deserve the reputation they have earned as cures. History shows that many of our important pharmacopoeial drugs were known and were also used in some form or other possibly long before they were introduced into the western medicine and before their actions were investigated on scientific lines. On the
other hand, there are sure to be others of little therapeutic value that are given only because they are mentioned in some old manuscripts, and no one has taken the trouble to confirm the truth of these statements. Attempts must be made to separate the good ones from the useless ones and for this a systematic investigation of these drugs must be undertaken.  

Much more could be done in furthering the cause of indigenous medicine and making it really useful to the people in this country by a thorough study of the indigenous drugs than by wholesale revivals of the old system under vastly changed environments. The economic condition of people is so low that they often can not afford to use the expensive medicines of the western system which are mostly imported from outside.  

The materia medica of the Indian system of medicine has been derived mainly from the vegetable kingdom. The knowledge about the use of medicinal plants has accrued through centuries and such plants are still valued today although synthetic drugs have come into more prominence in modern science. All living organisms ultimately derive energy from the sun. In a highly complex manner man can utilize certain but not all of the carbohydrate from cereal grains represent
the primary source of energy of the world’s population. Unlike fats and proteins carbohydrates are present in the body in the form of glycogen. The chief function of carbohydrates serve as the starting point for the synthesis of certain amino acids when a source of nitrogen is also available. The liberal use of carbohydrate food is advantageous because they are easily digested and almost completely absorbed for efficient use in producing energy, they are protein sparing in action. When selected from enriched and whole grains sources or potatoes the B-complex vitamin and iron intakes are appreciably increased and they are widely distributed and economical.146

Carbohydrates are constituents of all forms of animal and plant life. A few specific carbohydrates such as glycogen and lactose are synthesized only in animals but the overwhelming majority occur in plants, where they are synthesized from carbon dioxide and water. They can be broadly classified into four main groups on the basis of molecular structure. The main classifications are monosaccharides, disaccharides, oligosaccharides and polysaccharides.

Carbohydrates constitute one of the three main classes of nutrition. They are found in all plants where they are produced from carbon dioxide and water. The high intake of
carbohydrate increases dental decay, it increases the possibility of coronary heart disease, it contributes in increases obesity because carbohydrates not required for energy is converted into fat.

It has been possible, by means of one- and two-dimensional paper chromatography to separate many common carbohydrates. Polysaccharides from medicinal plants are hydrolyzed and the hydrolyzates are treated like other simple carbohydrate mixtures.

Most of the methods described in determination of aminoacids are being successfully applied to the carbohydrates. One- and two-dimensional paper chromatography methods are the most popular techniques for the carbohydrates also. 147
DISCUSSION

Systematic investigation of medicinal plants used in indigenous medicine in India on modern scientific lines was started from early twenties. A number of important medicinal plants prescribed by "Ravirajas" and "Hakims" have been investigated. The constituents have been examined, pharmacological action of the active principles worked out by animal experimentation. A large number of drugs examined has been shown to possess significant activity. Many drugs of questionable value and doubtful utility have crept into indigenous medicine and these have to be excluded after investigation.

The scientific mind is not satisfied by mere statements and traditions, no matter from what source they originate, unless corroborated by clinical and experimental evidences.

The descending or ascending method is generally used. Two-dimensional chromatography of a mixture of Xylose, Arabinose, Mannose, Glucose and Galactose is carried out in one-dimensional apparatus. Good separation of Lactose, Maltose, Sucrose, Glucose and Mannose is achieved in 72 hours. This effective increase of separation can be achieved by multiple development.

Quantitative determination of sugars can be done after
development of colour spots on paper chromatograms. A remarkable advance in Quantitative estimation of mixtures of sugars have been done by paper chromatographic technique.120

The qualitative and quantitative analysis of carbohydrates from dried medicinal plant samples were carried out after hydrolysis, by two-dimensional paper chromatography using phenol:water 90:10 v/v (NH₃ atmosphere) as first solvent and n-butanol:acetic acid:water (4:1:5) as second solvent. Aniline hydrogen phthalate was used as developing reagent.148

Rao and Gakhar149 studied tubers of Asparagus racemosus Willd. The hydrolysed fractions show only Glucose and Galacturonic acid. Landge, Landge and Bose150 studied ethanolic extract of roots of Asparagus racemosus Willd and yielded D-Glucose, D-Mannose and a new disaccharide. Sharma et al151 studied the fruits of Asparagus racemosus Willd and shown the presence of D-Glucose and L-Rhamnose. Gupta and Sharma152 studied sugars in leaves of Vitex negundo Linn and showed the presence of Glucose and Rhamnose only.

Our analysis showed following sugars in selected medicinal plants.

Alhagi camelorum Fisch showed L-Sorbose (0.500), L-Fucose (1.200), L-Rhamnose (0.800), D-Galacturonic acid
(1.500), D-Glucose (0.400) and D-Arabinose (1.300) sugars in gram per 100 gram of medicinal plant.

Crataeva religiosa Hook showed Lactose (0.550), D-Galactose (1.100), L-Sorbose (0.620), L-Fhamnose (0.250), D-Arabinose (0.810) and Sucrose (0.950) sugars in gram per 100 gram of medicinal plant.

Asparagus racemosus Willd showed Lactose (0.500), Maltose (0.100), D-Ribose (0.050), D-Mannose (0.320) and D-Fructose (0.550) sugars in gram per 100 gram of medicinal plant.

Swertia chirata Ham showed Lactose (0.200), L-Fucose (0.070), Maltose (0.100), D-Arabinose (0.030), D-Xylose (0.250), D-Mannose (0.050) and D-Fructose (0.100) sugars in gram per 100 gram of medicinal plant.

Vernonia anthelmintica Willd showed Lactose (0.010), D-Galactose (0.120), D-Arabinose (0.025), D-Mannose (0.110) and D-Fructose (0.045) sugars in 100 gram of medicinal plant.

Holarrhena antidysentrica Wall showed Lactose (0.300), D-Galactose (0.150), L-Sorbose (0.050), Maltose (0.150), D-Glucose (0.350), D-Arabinose (0.250) and D-Fructose (0.150) sugars in gram per 100 gram of medicinal plant.

Piper nigrum Linn showed L-Fucose (0.010), Maltose (0.020), D-Arabinose (0.130), D-Xylose (0.045) and D-Fructose
(0.035) sugars in gram per 100 gram of medicinal plant.

Finally Vitex negundo Linn showed D-Galactose (1.250), L-Fucose (2.600), D-Glucose (1.750), D-Arabinose (1.910) and Sucrose (1.670) sugars in gram per 100 gram of medicinal plant.
D-GALACTURONIC-ACID

D-GLUCOSE
MALTOSE

D-MANNOSE
### TABLE VI

**QUALITATIVE AND QUANTITATIVE ANALYSIS OF SUGARS OF SOME MEDICINAL PLANTS.**

**GM OF SUGARS/100 GM OF MEDICINAL PLANTS.**

<table>
<thead>
<tr>
<th>No</th>
<th>Sugars</th>
<th>Alhagi camel-rum Fisch</th>
<th>Crataeva religiosa Hook</th>
<th>Asparagus racemosus Wild</th>
<th>Swertia chirata Ham</th>
<th>Vernonia anthelminthica Wild</th>
<th>Holarrheana antidysenterica Wall</th>
<th>Piper nigrum Linn</th>
<th>Vitex negundo Linn</th>
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<tbody>
<tr>
<td>1</td>
<td>Lactose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>D-Glucosamine HCl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>D-Galactose</td>
<td>-</td>
<td>1.100</td>
<td>-</td>
<td>0.120</td>
<td>0.150</td>
<td>-</td>
<td>1.250</td>
<td>-</td>
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<tr>
<td>4</td>
<td>L-Sorbose</td>
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<td>0.620</td>
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<td>-</td>
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</tr>
<tr>
<td>5</td>
<td>L-Fucose</td>
<td>1.200</td>
<td>0.250</td>
<td>-</td>
<td>-</td>
<td>0.050</td>
<td>-</td>
<td>-</td>
<td>2.600</td>
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<tr>
<td>6</td>
<td>L-Rhamnose</td>
<td>0.800</td>
<td>0.100</td>
<td>0.100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>7</td>
<td>Maltose</td>
<td>1.500</td>
<td>0.100</td>
<td>0.100</td>
<td>0.150</td>
<td>0.020</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>8</td>
<td>D-Galacturonic acid</td>
<td>0.400</td>
<td>0.810</td>
<td>0.030</td>
<td>0.025</td>
<td>0.250</td>
<td>0.130</td>
<td>1.910</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>D-Glucose</td>
<td>1.300</td>
<td>0.810</td>
<td>0.030</td>
<td>0.025</td>
<td>0.250</td>
<td>0.130</td>
<td>1.910</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>D-Arabinose</td>
<td>-</td>
<td>-</td>
<td>0.250</td>
<td>-</td>
<td>-</td>
<td>0.045</td>
<td>-</td>
<td>-</td>
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<tr>
<td>11</td>
<td>D-Xylose</td>
<td>-</td>
<td>-</td>
<td>0.050</td>
<td>-</td>
<td>-</td>
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<tr>
<td>12</td>
<td>D-Ribose</td>
<td>-</td>
<td>0.950</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.870</td>
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<tr>
<td>13</td>
<td>Sucrose</td>
<td>-</td>
<td>0.320</td>
<td>0.050</td>
<td>0.110</td>
<td>-</td>
<td>-</td>
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<tr>
<td>14</td>
<td>D-Mannose</td>
<td>-</td>
<td>0.550</td>
<td>0.100</td>
<td>0.045</td>
<td>0.150</td>
<td>0.035</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>D-Fructose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>Total Sugars</td>
<td>5.700</td>
<td>4.280</td>
<td>1.520</td>
<td>0.800</td>
<td>0.310</td>
<td>1.400</td>
<td>0.240</td>
<td>9.180</td>
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STANDARD CHART OF SUGARS
ALHAGI CAMELORUM FISCH

SOLVENT-I

SAMPLE-A

SOLVENT-II

SOLVENT-I

SAMPLE-B

SOLVENT-II

SOLVENT-I

CRATAEVA RELIGIOSA HOOK
PIPER NIGRUM LINN

SAMPLE-G

SOLVENT-II

SOLVENT-I

VITEX NEGUNDO LINN

SAMPLE-H

SOLVENT-II

SOLVENT-I
Wild Asparagus (Asparagus racemosus)

Three Leaved Caper (Crataeva religiosa)

Camel Thorn (Alhagi camelorum)

Wild Asparagus (Asparagus racemosus)

Chireta (Swertia chirata)
EXPERIMENTAL

The medicinal plants which have been taken for aminoacid analysis were further studied for qualitative and quantitative determination of sugars. For this purpose, the paper chromatography technique was carried out.

DETERMINATION OF HRF VALUES OF SEVERAL SUGARS:

In order to identify the spots of various sugars developed on a paper chromatogram it is essential to determine hRF values of several sugars with respect to the solvent system. The paper used in this investigation was Whatman No.1 filter paper (8x8 inches). Lines one inch away from both the edges were drawn by a pencil. The solutions of common 15 sugars were prepared separately by dissolving 1 gm sugar in 100 ml of hot distilled water.

Fifteen sugars were divided into four groups as below:

Group-A: L-Sorbose, D-Glucose, L-Rhamnose, D-Ribose.

Group-B: D-Galacturonic acid, D-Galactose, Lactose, Sucrose.

Group-C: Maltose, D-Xylose, D-Glucosamine-HCl, D-Arabinose.
Group-D: D-Mannose, L-Fucose, D-Fructose.

Micro quantity of each group of solution (previously mixed) were applied on to the left hand corner of four filter papers where the markings were already made. The drop of the solution when applied to filter paper will produce a circular spot due to absorption of the solvent on the Whatman filter paper. The application of the solution was made in such a way that the spot did not expand more than a circle with 0.5 cm in diameter. The spot was dried by means of electrical hair drier. The spot of four groups of sugars solution on four different papers were dried, the papers were placed in the chromatographic rack, which was later placed in a closed air tight chamber for chromatography.

The first solvent system phenol: water (90:10 v/v) (NH₃ atm) was taken into the trough in such a way that the lower edge of the chromatographic papers dip in the solvent system. The solvent was allowed to ascend up to 16 cm. Then, the rack containing chromatographic papers was removed from the chamber, and was allowed to dry over-night. The above process was repeated with the same solvent in the same edge on the second day to get clear distinguished spots of sugars on the third day, for two-dimensional chromatography. the rack
containing chromatographic papers was turned at right angle and kept in another chamber containing second solvent system n-Butanol:Acetic acid:Water (4:1:5). In the preparation of this solvent system, three components were mixed, shaken well and allowed to settle in separating funnel for half an hour. The upper n-butanol layer saturated with water was used for chromatography. The solvent was allowed to ascend up to 16 cm. When the solvent reached this point, the rack was removed from the tank and was allowed to dry over-night. On the fourth day the rack was again kept in the same solvent system at the same edge for second run. After drying, the paper were kept over-night, then they are sprayed with Aniline hydrogen phthalate reagent. The four chromatographic papers were hanged in air, slightly heated by hair-drier to get full development of coloured spots of sugars. The hRF value was determined by the formula:

$$hRF = \frac{\text{distance travelled by the spot}}{\text{distance travelled by the solvent}} \times 100$$

The distances were measured in cm from the point of application of sugar solution. The hRF values were calculated for both the solvent systems. The position of the spots, hRF values and the colour of the spots were used for identifying
the unknown sugars present in selected medicinal plants.

STANDARDIZATION OF SUGARS PRESENT IN MEDICINAL PLANTS FOR QUANTITATIVE ANALYSIS

For quantitative estimation of the sugars detected in the medicinal plants the weighed quantity of sugars were dissolved in hot distilled water. Individual sugars were spotted using a micropipette on individual Whatman No.1 filter paper in the amount 0.02 ml, 0.04 ml, 0.06 ml, 0.08 ml and 0.1 ml at one corner, leaving an inch from both the sides.

Two-dimensional chromatography was carried out as above using phenol:water (90:10 v/v) as the first solvent system and n-butanol:acetic acid:water (4:1:5) as the second solvent system. After chromatography the papers were dried and sprayed with Aniline hydrogen phthalate solution. After full development of the coloured spots on the papers, they are cut and placed in separate test tubes containing water.

To 1-5 ml of sugar solution equal amount of alkaline copper reagent is added. Sample solution is heated for 10 minutes in a vigorously boiling water bath and then cooled, one ml of arsenomolybdate reagent is added to determine amount of sugar, when all the cuprous oxide is dissolved.
after mixing the solution it is diluted to 25 ml mark on the tube and allowed to stand at least 15 minutes but not more than 40 minutes. Absorbance was read at 500 μm in the spectrophotometer.

Similar treatment is also given to the blank paper and blank reading is subtracted from the sample reading.

The graph plotted is optical density versus ml of sugar solution taken. Thus standard graphs of different sugars are drawn.

**EXTRACTION OF SUGARS FROM MEDICINAL PLANTS:**

Finely powdered (1 gm) dried sample of the medicinal plant was dropped in 50 ml of 80% boiling ethanol, and extraction was carried out under reflux soxlet condenser for about 12 hours. Then the solution was evaporated on water bath, care should be taken that evaporation is not up to complete dryness. This solution was clarified with decolouring charcoal until the solution becomes clean.

This clear solution was further subjected to purification for removal of impurities such as aminoacids and other natural elements. Add minute quantity of saturated solution of lead acetate to cause complete precipitation of
impurities. This was filtered by Whatman No.1 filter paper. Filtrate was then subjected to further analysis.

**LOW-ALKALINITY COPPER REAGENT**\(^1\)\(^\text{156}\)

Rochelle Salt (12 gm) and anhydrous sodium carbonate (24 gm) are dissolved in about 250 ml of water. A solution of 4 gm of cupric sulphate pentahydrate in water is added with stirring followed by 16 gm of sodium hydrogen carbonate. A solution of 180 gm of anhydrous sodium sulphate in 500 ml of water is boiled to expel air; then the two solutions are combined and diluted to 1 litre. After one week of standing, the clear supernatant solution is used.

**ANILINE HYDROGEN PHTHALATE**\(^1\)\(^\text{157-158}\)

The reagent is prepared by adding aniline (0.93 gm) and phthalic acid (1.66 gm) to water saturated butanol (100 ml) and after spraying, the chromatogram is heated for 5 minutes at 105°c. The aldopentoses give a bright red colour, while the aldohexoses give brown colour.